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(54) Title: METHOD FOR THE PREPARATION OF FACIAL METAL TRICARBONYL COMPOUNDS AND THEIR USE IN THE LABELLING OF BIOLOGICALLY ACTIVE SUBSTRATES

(57) Abstract

The invention relates to a method of preparing a compound of the general formula (I): *fac*-$[M(CO)_3(OH_2)_3]^+$ wherein M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re, by reacting a metal in the permetallate form with carbon monoxide and a reducing agent, characterized in that a mixture of a base, a reducing agent soluble in water but not substantially decomposed by water, and optionally a stabilizing agent is solved in a water containing solvent system containing a solution of the metal in the permanganate, pertechnetate or perrhenate form in the presence of carbon monoxide and optionally in the presence of a halide. The invention further relates to a method of preparing a labeled compound with the aid of the compound of general formula (I), to a method of direct preparation of labeled compounds, to a method of labeling of substrates such as amino acids, peptides, proteins, sugars, small receptor binding molecules and body cells with the aid of compound of general formula (I) and to a kit for the preparation of a labeling composition and a kit for the preparation of a diagnostic of therapeutic pharmaceutical composition.

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Method for the preparation of facial metal tricarbonyl compounds and their use in the labelling of biologically active substrates

The invention relates to a method of preparation of facial metal tricarbonyl compounds and further co-ordinated facial metal tricarbonyl compounds. The invention further relates to the use of said facial metal tricarbonyl compounds in the labelling of biologically active substrates and other ligands, and to a kit for preparing a facial metal tricarbonyl compound or further co-ordinated facial metal tricarbonyl compounds.

The application of metal complexes, with a wide variety of radionuclides, in the field of nuclear medicine has become a major tool in diagnosis and also more recently in therapy. The metal complexes are often attached to a biologically active substrate that acts as a targeting agent. One of the most widely applied procedures for the metal-labelling of biologically active substrates such as proteins, peptides, sugars or small biologically active compounds consists in stabilizing the M(V)=O moiety of (radioactive) metals of group 7B of the periodic table with different tetradentate ligands. After reduction, the M(V)=O moiety is intermediately stabilized with a larger amount of an auxiliary ligand such as glucoheptonate which is subsequently substituted by the chelator attached to the system to be labelled. This method has proven to be successful in many cases but suffers from some major disadvantages such as the required high denticity and the bulkiness of the ligand and the difficulty in synthesizing and attaching such ligand.

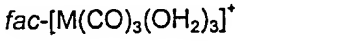
It is known in the art (Alberto et al., J. Nucl. Biol. and Med. 1994, 38, 388-90) that facial metal tricarbonyl complexes of radioactive metals of group 7B of the periodic table are very convenient starting materials for substitution reactions in organic solvents as well as in water, as these compounds are stable in water for weeks, even if exposed to air. Therefore said compounds would be very useful for the labelling of biologically active substrates, such as amino acids, peptides, proteins, sugars and any receptor binding molecules. A major drawback, however, of these compounds until now is that they have only been available from high temperature carbonylation reactions and with the aid of the pyrophoric and toxic and therefore dangerous reducing agent BH_3 (Alberto et al., Low CO pressure synthesis of $(NEt)_2[MX_3(CO)_3]$ (M = Tc, Re) and its Substitution Behaviour in Water and Organic

Solvents. Technetium in Chemistry and Nuclear Medicine, No 4, Cortina International, Milano, 1994).

It is the objective of the present invention to provide for a method of preparing facial metal tricarbonyl compounds of (radioactive) metals of group 7B with the aid of easily available and low-toxic starting materials at moderate temperature and at normal pressure of CO, in a reasonable time and with high yield.

Such a method would be a powerful tool that can be used for the synthesis of diagnostic and therapeutic agents, especially for the synthesis of said diagnostic and therapeutic agents derived from radioactive metals with a short lifetime, in order to have access to these labelled compounds in poorly-equipped hospital laboratories. When the above mentioned diagnostic agent is labelled with a radionuclide it can be detected by the so-called single photon emission computerized tomography (SPECT and SPET), when it is labelled with a paramagnetic metal atom it can be detected by magnetic resonance imaging.

The above-defined objective can be achieved, according to the present invention, by a method of preparing a compound of the general formula



fac-$[M(CO)_3(OH_2)_3]^+$ (I)

wherein M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re,

by reacting a metal in the permetallate form (MO_4^- form) with carbon monoxide and a reducing agent, characterized in that a mixture of a base, a reducing agent soluble in water but not substantially decomposed by water, and optionally a stabilizing agent is solved in a water containing solvent system containing a solution of the metal in the permanganate, pertechnetate or perrhenate form in the presence of carbon monoxide.

The metal M is preferably ^{99m}Tc, ^{186}Re or ^{188}Re, as these radionuclides, when used in diagnostic or therapeutic agents, have the advantage that they can be applied in very low concentrations, which minimizes the risk of toxicity.

The term "not substantially decomposed by water" means that upon the addition of the solution of permanganate, pertechnetate or perrhenate in water, the velocity of the decomposition reaction of the reducing agent with water is zero or very low

compared with the reaction of said reducing agent with the permanganate, pertechnetate or perrhenate, so that the reaction with said permetallate is completed when still enough of the reducing agent is present.

It is very surprising that a quantitative reduction of permetallates in water containing solvent systems can be achieved at moderate temperature and in reasonable times with reducing agents that are nucleophilic and that are generally considered as less reactive than the electrophilic reducing agent BH_3 known in the art.

The method of the invention can be easily performed just by mixing the permetallate solution with the other reagents in the presence of carbon monoxide. The permetallate solution may optionally contain halide ions needed for the elution of the permetallate from a generator. The carbon monoxide may be supplied by using a closed system with an atmosphere containing a sufficient amount of carbon monoxide, or by flushing the carbon monoxide gas through the solution.

The base used is preferably an inorganic base, selected from the group of stable hydroxides and carbonate salts such as NaOH, KOH, $NaHCO_3$, Na_2CO_3, $KHCO_3$, K_2CO_3, $Ca(OH)_2$ and $Mg(OH)_2$. Most preferred is Na_2CO_3. The base is added in a molar ratio to the reducing agent of between 0.1 and 2, and preferably in a molar ratio of approx. 0.35 .

The reaction can be performed with and without a stabilizing agent. As a stabilizing agent gentisate (2,5-dihydroxybenzoate), glucoheptonate, citrate or tartrate can be used. The preferred stabilizing agent is tartrate, e.g. as NaK-tartrate. The stabilizing agent is added to the reaction mixture in such an amount that its concentration is higher than that of the metal to be reduced.

For the reduction several reducing agents can be used, such as borohydride anion (BH_4^-) or substituted borohydride anion wherein up to three of the hydrogen atoms which comprise the borohydride anion have been independently replaced by inert substituents. Examples of said inert substituents are alkoxy or alkylcarbonyloxy groups containing 1 to 10 carbon atoms and cyano groups. The counterion of the reducing group may consist of a metal of group 1A or 2A of the periodic table or zinc or an ammonium or tetrasubstituted ammonium or tetrasubstituted phosphonium ion, wherein the four substituents are each independently alkyl groups containing from 1 to 10 carbon atoms, hydroxyalkyl groups or alkoxyalkyl groups containing from 2 to 10 carbon atoms or aryl groups,

Preferred reduction reagent is borohydride anion, especially in the form of compounds such as sodium borohydride, potassium borohydride, lithium borohydride and zinc borohydride. Most preferred reducing agent is sodium borohydride.

The reducing agent is reacted with the permetallate in a molar ratio higher than 3. The reduction reaction can be performed at a temperature between 20°C and 100°C. The preferred reaction temperature is approx. 75°C. The heating of the reaction mixture can be performed in the normal way but also by micro-wave heating. The reaction can also be performed by the application of ultra sound, e.g. by carrying out the reactions in an ultrasonic bath at room temperature, normally leading to the same reaction rate at lower reaction temperature.

The compound of the general formula (I) obtained is very suitable for the labeling of biologically active substrates, such as amino acids, peptides, proteins, sugars, small receptor binding molecules or cells.

Examples of peptides that may be labelled are growth factors, somatostatin, bombesin, insulin, LHRH, gastrin, gastrin releasing peptide, thyrotropin releasing hormone, thyroid stimulating hormone, prolactin, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), angiotensin, neurotensin, interferons, IL-1, IL-4 and IL-6, monoclonal antibodies and their analogues and derivatives. After labelling with a suitable labelling substance these peptides can e.g. be used in the detection and localisation or treatment of malignant human tumours.

Examples of sugars that may be labelled are glucose and deoxyglucose and derivatives of said compounds.

Small receptor binding molecules are defined as non-peptide molecules which are binding to a receptor and normally have a molecular mass below approximately 500 Daltons.

Examples of small receptor binding molecules that may be labelled are substances for the serotonergic system as described in WO 96/30054, or substances for the dopaminergic system (e.g. raclopride, β-CIT, lisuride), for the cholinergic system (e.g. epibatidine), for the glutaminergic system (e.g. memantine) or for the benzodiazepine system (e.g. flumazenil, iomazenil). Examples of metabolic active

molecules that may be labelled are DOPA, Tyrosine, mIBG, MAO-I and analogues thereof.

Examples of cells that may be labelled are red and white blood cells.

As a result of the labeling of (biologically active) substrates with a compound of the general formula I, a further coordinated compound of the general formula

fac-$[M(CO)_3(X)_2L_1]^n$ (II),
fac-$[M(CO)_3(X)L_2]^n$ (III) or
fac-$[M(CO)_3L_3]^n$ (IV),

wherein:

M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re;

L_1 is a monodentate ligand,

L_2 is selected from the group consisting of a bidentate ligand and two monodentate ligands, and

L_3 is selected from the group consisting of a tridentate ligand, a monodentate ligand and a bidentate ligand, and three monodentate ligands;

X is H_2O or a halide ion;

n the sum of the charge of the ligands L_1 or L_2 or L_3 and X increased with one + charge

is obtained.

After the labeling reaction the ligand X is usually H_2O. One of the H_2O ligands may, however, be replaced by a halide ion, when available, to neutralize the charge of the complex. This is often the case for compounds of the general formula III.

When the ligand L_1, L_2 or L_3 before and/or after labeling with the facial metal tricarbonyl compound is the biologically active molecule, the present invention gives easy access to compounds that directly can be used as a diagnostic and therapeutic agent.

Examples of monodentate ligands within the definition of L_1, L_2 and L_3 are (biologically active) substrates bearing groups such as phosphines, isonitriles, nitriles, imidazoles, thioethers and pyridine-like aromatic amines.

Examples of bidentate ligands within the definition of L_2 and L_3 are (biologically active) substrates bearing pyridin, imidazole or pyrazole groups, such as histidine, histamine, functionalized imidazole systems, bidentate thioethers, bidentate isocyanides, Schiff-base type ligands and picolinic acid.

Examples of tridentate ligands within the definition of L_3 are tris-pyrazolyl borate, tris-pyrazolylmethane, tris-imidazolyl borate, tris-pyrazolylmethane, 1,4,7-trithiacyclononane (9-aneS_3) and triazacyclononane (9-aneN_3), histidine, methionine, cystein derivatized at the thiol group to give a thioether and cyclopentadienyl derivatives.

In some cases it may be advantageous to prepare the radiolabelled bioactive compound in one step. This objective can be achieved according to the present invention, with a method of preparing a compound of the general formula

fac-$[M(CO)_3(X)_2L_1]^n$	(II),
fac-$[M(CO)_3(X)L_2]^n$	(III) or
fac-$[M(CO)_3L_3]^n$	(IV),

wherein:

M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re;

L_1 is a monodentate ligand,

L_2 is selected from the group consisting of a bidentate ligand and two monodentate ligands, and

L_3 is selected from the group consisting of a tridentate ligand, a monodentate ligand and a bidentate ligand, and three monodentate ligands;

X is H_2O or a halide ion;

n the sum of the charge of the ligands L_1 or L_2 or L_3 and X increased with one + charge;

characterized in that a mixture of a base, ligands L_1 or L_2 or L_3, a reducing agent soluble in water but not substantially decomposed by water, and optionally a

stabilizing agent is solved in a water containing solvent system containing a solution of the metal in the permanganate, pertechnetate or perrhenate form in the presence of carbon monoxide and optionally in the presence of halide.

Especially in the case of radiolabelled compounds it is frequently impossible to put the ready-for-use composition at the disposal of the user, in connection with the often poor shelf life of the radiolabelled compound and/or the short half-life of the radionuclide used. In such cases the user will carry out the labelling reaction with the metal in the clinical hospital or laboratory. For this purpose the various reaction ingredients are then offered to the user in the form of a so-called "kit". It will be obvious that the manipulations necessary to perform the desired reaction should be as simple as possible to enable the user to prepare from the kit the radioactive labelled composition by using the facilities that are at his disposal. Therefore the invention also relates to a kit for preparing a labelling composition, which labelling composition contains compound of formula I as the labelling agent.

Such a kit for the labelling of a biologically active substrate, according to the present invention, comprises (i) a reducing agent soluble in water but not substantially decomposed by water, (ii) a base, (iii) if desired, a stabilizing agent and/or a chelator and (iv) if desired one or more inert pharmaceutically acceptable carriers and/or formulating agents and/or adjuvants, at least one of said ingredients (i) to (iv) being stored in a container having an atmosphere containing a sufficient amount of carbon monoxide, said ingredients (i) to (iv) optionally independently being combined, and (v) instructions for use with a prescription for reacting the ingredients of the kit with a metal selected from the group consisting of Mn, ^{99m}Tc, ^{186}Re or ^{188}Re in the form of a permetallate solution.

It is the merit of the present invention, disclosing an easy way of preparing facial tricarbonyl metal compounds within a time-frame that is reasonable compared with the half-life time of the radioactive isotopes involved, and with high yields, that a kit can be prepared for the labelling of biologically active substrates with said facial tricarbonyl metal compounds.

In some cases it may be advantageous to enclose a bioactive substrate in the kit so that a kit is obtained for the preparation of a radiopharmaceutical composition.

Alternatively the biologically active compound is formed upon the reaction of the ligand with the facial metal tricarbonyl compound.

Such a kit for the preparation of a diagnostic and therapeutic pharmaceutical composition, according to a different embodiment of the present invention, comprises (i) a suitable substrate to be labelled with a metal selected from the group consisting of Mn, ^{99m}Tc, ^{186}Re or ^{188}Re, (ii) a reducing agent soluble in water but not substantially decomposed by water, (iii) a base, (iv) if desired, a stabilizing agent and/or a chelator, (v) if desired one or more inert pharmaceutically acceptable carriers and/or formulating agents and/or adjuvants, at least one of said ingredients (i) to (v) being stored in a container having an atmosphere containing a sufficient amount of carbon monoxide, said ingredients (i) to (v) optionally independently being combined, and (vi) instructions for use with a prescription for reacting the ingredients of the kit with said metal in the form of a permetallate solution.

The preparation of the diagnostic and therapeutic pharmaceutical composition with the aid of the above mentioned kit enclosing a (biologically active) substrate can take place in two alternative embodiments. In the first embodiment the facial tricarbonyl metal compound is prepared first and then reacted with the substrate to be labelled. In the second embodiment the reduction step is carried out in the presence of the substrate to be labelled, directly leading to the labelled compound.

The invention will now be described in greater detail with reference to the following specific Examples.

Example 1. Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from aqueous solution

In a 10 ml closable vial the following chemicals are put together: 5.5 mg of $NaBH_4$, 4.0 mg Na_2CO_3 and 20.0 mg NaKtartrate. The vial is closed with a serum stopper and flushed for 10 minutes with carbon monoxide gas with the aid of a syringe. 3 ml of a 0.9% NaCl solution from a Mo-99/Tc-99m generator, having an activity of about 100 mCi, is added via the septum and the vial is heated to 75°C during 30 minutes and then cooled to room temperature. The product is analysed by TLC on standard Merck silica gel plates with methanol/conc. HCl = 99/1 as mobile phase followed by analysis of the silica gel plate by means of a radioactivity scanner. The yield of the reduction of pertechnetate to facial $[^{99m}Tc(OH_2)_3(CO)_3]^+$ is > 95%

according to TLC. After neutralizing the solution with a solution of PBS (phosphate buffer (pH = 7.4, saline 0.9%)) a neutral physiological solution, suitable for labelling is obtained.

Table 1 shows that solutions of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ having an activity up to 700 mCi can be obtained under different reaction conditions.

Table 1. Preparation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ under different reaction conditions.

Exp.	Stabilizing agent	Volume TcO_4^- sol. (ml)	Activity (mCi)	Solvent	Temp. (°C)	React. time (min)	Yield (TLC) (%)
1	NaKtartrate	3	≈ 100	H_2O	75	30	> 95
2	NaKtartrate	3	≈ 400	H_2O	75	30	> 95
3	NaKtartrate	3	≈ 700**	H_2O	75	30	> 95
4	NaKtartrate	3	n.d.*	H_2O	75	30	> 95
5	NaKtartrate	6	n.d.*	H_2O	75	30	> 95
6***	NaKtartrate	3	n.d.*	H_2O	75	30	40
7	-	3	n.d.*	H_2O	75	30	70
8	Nacitrate	3	n.d.*	H_2O	75	30	20
9	Naformiate	3	n.d.*	H_2O	75	30	35
10	NaKtartrate	3	n.d.*	H_2O/EtOH 80/20	75	30	> 95
11	NaKtartrate	3	n.d.*	H_2O	100	10	80

* Activity not determined exactly, but always between 50 and 200 mCi.

** Activity determined after dilution to 1%

*** 4.0 mg $Ca(OH)_2$ has been used as a base instead of 4.0 mg Na_2CO_3

Example 2. Synthesis of complexes of composition $[^{99m}TcL(CO)_3]$

2.1 Synthesis of $[^{99m}Tc(his)(CO)_3]$ via $[^{99m}Tc(OH_2)_3(CO)_3]^+$.

After completion of the reaction as described in Example 1, 0.1 μmol of histidine is added to the solution of pH 7.4. According to TLC the reaction is complete after 1 hour.

When 0.01 μmol of histidine is added at room temperature, the reaction takes 5-10 hours before completion; the reaction is completed in less than 1 hour at 70°C.

2.2 Direct synthesis of $[^{99m}Tc(his)(CO)_3]$.

The experiment is carried out as described in Example 1. Concerted to the addition of the generator eluate to the cold vial, 0.03 µmol of histidine is added to said cold vial. After heating during 30 minutes $[^{99m}Tc(his)(CO)_3]^+$ is obtained in almost quantitative yield according to TLC.

2.3 Synthesis of $[^{99m}Tc((lys\text{-}gly\text{-}(his)_5)(CO)_3]^+$ via $[^{99m}Tc(OH_2)_3(CO)_3]^+$.

After completion of the reaction as described in Example 1, 500 pmol of the octapeptide lys-gly-gly-$(his)_5$ is added to the solution. According to TLC the reaction is complete after 1 hour at room temperature.

When 300 pmol of lys-gly-gly-$(his)_5$ is added the reaction takes 3 hours to complete.

2.4 Summary of preparation of further complexes of composition $[^{99m}TcL(CO)_3]$

The $[^{99m}Tc(OH_2)_3(CO)_3]^+$ is prepared as described in Example 1 and neutralized. The ligand (see Figure 1) is added with subsequent complexation time and temperature and in amount and concentration as indicated in Table 2. Table 2 also indicates the yields obtained as determined by TLC analysis as described in Example 1 and the possibility of carrying out the reaction in a one pot process.

Table 2. Preparation of $[^{99m}TcL(CO)_3]$ with different ligands and under different reaction conditions.

Ligand (Fig.1)	pH.	time	temp	Conc.	absolute amount	yield	one pot ****
1	PBS	30'	70° C	3 µM	5 nmol	92 %	partially
2	PBS	2-3 h	37°C	20 µM	10 nmol	74 %	yes
3	PBS	4 h	37°C	100 µM	50 nmol	89 %	yes
4	PBS	6 h	37°C	100 µM	50 nmol	72 %	-
5	PBS/ CH_3OH	1 h	50°C	100 µM	100 nmol	65 %	-
6	PBS	1h+ 1h	50°C	*		97%+ 30%	-
7***	PBS	30'	75°C	25 µM	25 nmol	95%	yes
7***	PBS	15'	**	25 µM	25 nmol	95%	yes
8	PBS	1h	75°C	10 µM	2 nmol	95%	yes

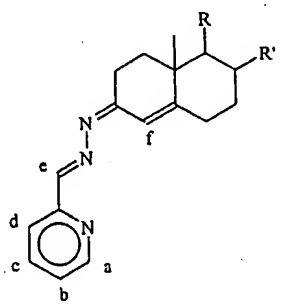
* 50µl 10^{-3}M ligand 1, then 50µl 10^{-2}M glucose

** 15 min ultra sound r.t.

*** After the reaction the product was stored for 23 h at 37°C and appeared to be stable

**** possibility to perform as a one pot synthesis

From the compound derived from ligand 5 (see structure below) the ^{1}H-NMR spectrum was determined before and after complexation with "cold" $[Tc(OH_2)_3(CO)_3]^+$. A: aromatic region of NMR spectrum before complexation: ^{1}H-NMR ($CDCl_3$)[r.t., δ in ppm] = 8.75 (1H, d)(a), 8.36 (1H, s)(e), 8.14 (1H, d)(d), 7.99 (1H, t)(b), 7.55 (1H, m)(c), 6.07 (1H, s)(f). B: aromatic region of NMR spectrum after complexation: ^{1}H-NMR ($CDCl_3$)[r.t., δ in ppm] = 9.12 (1H, d)(a), 8.51 (1H, s)(e), 8.37 (1H, t)(b), 8.19 (1H, d)(d), 7.87 (1H, m)(c), 6237 (1H, s)(f).



Example 3. Labelling of antibodies with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

3.1 Labelling as function of concentration.

Labelling kinetics is tested as a function of Mab concentration. Concentration above 2-3 mg/ml yields quantitative labelling after 2 hours, whereas below 1 mg the yield is about 40-50% according to TLC.

3.2. In vitro biological activity of labelled monoclonal antibody 35 (Mab-35)

An amount of $[^{99m}Tc(OH_2)(CO)_3]^+$ as prepared in Example 1 is used for labelling of 1.2 mg of Mab-35. After 3 hours of incubation at 37 °C, the Mab is separated over a PD-10 size exclusion gel chromatography column with 38% yield. The labelled Mab is brought to a Lindmo testing (T. Lindmo, P.A. Brunn, Methods in Enzymology 1986, 121, 678), showing 100% biological activity.

Example 4. Labelling of His-Neurotensin(8-13) with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

0.9 ml of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ as prepared in example 1 is mixed with 0.1 ml of 10^{-3}M His-Neurotensin(8-13) (HRRPYIIL) and kept in a sealed tube at 75°C for one hour. After this time the reaction mixture is cooled to room temperature. As evident from reversed phase column HPLC the yield is >95%. The K_d of this compound on coloncarcinoma cells HT29 is 1.0 nM.

Example 5. Labelling of the protein fragment recombinant scFv with 6xHis-tag with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

0.1 ml of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ as prepared in Example 1 is mixed with 0.1 ml of 1M MES-buffer pH 6.2 and 0.1 ml of 150 µM scFv-6xHis and kept at 37°C for 20 min. After this time the reaction mixture is separated on a Sephadex® G-25 Superfine sizing column. Typical incorporations of ^{99m}Tc are 70% to 84%, with biological activities (measured by the Lindmo testing mentioned in Example 3) ranging from 57% to 90%. K_d values were not significantly altered by the ^{99m}Tc incorporation: measurement by BIAcore of the unlabeled scFv: $0.5x10^{-8}$M, ^{99m}Tc-labelled scFv: $1x10^{-8}$M, ^{125}I-labelled scFv: $4x10^{-8}$M.

Example 6. Labelling of biotin with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

$[^{99m}Tc(OH_2)_3(CO)_3]^+$ is prepared as described in example 1. 1.300µl of a 10^{-3}M biotin-hydrazide-pyridine solution is added to 2 ml of the Tc-carbonyl compound and is incubated at 50°C for 2 hours to yield a Tc labelled compound with a purity

of 50%. The compound is purified over an equilibrated (5 ml MeOH/H_2O=1/1) SepPac column by loading and eluting by-products with 2 ml H_2O and then with 4 ml MeOH/H_2O=1/1 followed by eluting the desired product with 2 ml of MeOH. Final purity of the compound is 98%. Stability control: No decomposition in methanol after 24 hours; 32% decomposition in PBS buffer after 24 hours. Results of binding test to Streptavidin-beads: 0.5 % unspecific binding and 81% specific binding.

Claims

1. Method of preparing a compound of the general formula

$$fac\text{-}[M(CO)_3(OH_2)_3]^+ \qquad (I)$$

wherein M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re,
by reacting a metal in the permetallate form with carbon monoxide and a reducing agent, characterized in that a mixture of a base, a reducing agent soluble in water but not substantially decomposed by water, and optionally a stabilizing agent is solved in a water containing solvent system containing a solution of the metal in the permanganate, pertechnetate or perrhenate form in the presence of carbon monoxide.

2. Method of preparing a compound of the general formula

$$fac\text{-}[M(CO)_3(X)_2L_1]^n \qquad (II),$$
$$fac\text{-}[M(CO)_3(X)L_2]^n \qquad (III) \text{ or}$$
$$fac\text{-}[M(CO)_3L_3]^n \qquad (IV),$$

wherein:

M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re;

L_1 is a monodentate ligand,

L_2 is selected from the group consisting of a bidentate ligand and two monodentate ligands, and

L_3 is selected from the group consisting of a tridentate ligand, a monodentate ligand and a bidentate ligand, and three monodentate ligands;

X is H_2O or a halide ion;

n is the sum of the charge of the ligands L_1 or L_2 or L_3 and X increased with one + charge;

characterized in that a compound of general formula I prepared as claimed in claim 1 is reacted with ligands L_1 or L_2 or L_3, optionally in the presence of a halide.

3. Method of preparing a compound of the general formula

fac-$[M(CO)_3(X)_2L_1]^n$ (II),

fac-$[M(CO)_3(X)L_2]^n$ (III) or

fac-$[M(CO)_3L_3]^n$ (IV),

wherein:

M is Mn, ^{99m}Tc, ^{186}Re or ^{188}Re;

L_1 is a monodentate ligand,

L_2 is selected from the group consisting of a bidentate ligand and two monodentate ligands, and

L_3 is selected from the group consisting of a tridentate ligand, a monodentate ligand and a bidentate ligand, and three monodentate ligands;

X is H_2O or a halide ion;

n is the sum of the charge of the ligands L_1 or L_2 or L_3 and X increased with one + charge;

characterized in that a mixture of a base, ligands L_1 or L_2 or L_3, a reducing agent soluble in water but not substantially decomposed by water, and optionally a stabilizing agent is dissolved in a water containing solvent system containing a solution of the metal in the permanganate, pertechnetate or perrhenate form in the presence of carbon monoxide and optionally in the presence of a halide.

4. Method according to claims 2-3, characterized in that the ligands L_1-L_3 are or are derived from biologically active substrates selected from the group consisting of amino acids, peptides, proteins, sugars, small receptor binding molecules and body cells.

5. Method of labeling according to claim 4, characterized in that the substrate is an amino acid, a peptide or a protein.

6. Method according to claim 1 or 3, characterized in that the base is an inorganic base, preferably selected from the group consisting of NaOH, KOH, $NaHCO_3$, Na_2CO_3, $KHCO_3$, K_2CO_3, $Ca(OH)_2$ and $Mg(OH)_2$.

7. Method according to claim 1, 3 or 6, characterized in that the molar ratio between the base and the reducing agent is between 0.1 and 2, and preferably is approx. 0.35.

8. Method according to claim 1, 3 or 6 - 7, characterized in that the reducing agent is selected from the group consisting of borohydride anion and substituted borohydride anion wherein up to three of the hydrogen atoms which comprise the borohydride anion have been replaced by inert substituents.

9. Method according to claim 8, characterized in that the reducing agent is a borohydride anion derived from of a salt selected from the group consisting of sodium borohydride, potassium borohydride, lithium borohydride and zinc borohydride.

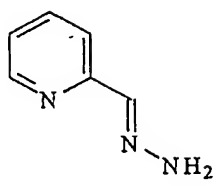
10. Method according to claim 9, characterized in that the reducing agent is sodium borohydride.

11. Method according to claim 10, characterized in that the molar ratio of the reducing agent to the permetallate is at least 3.

12. Method according to claims 1, 3 and 6-11, characterized in that the reaction time is between 20°C and 100°C and preferably is approx. 75°C.

13. A kit for the preparation of labelling composition, comprising (i) a reducing agent soluble in water but not substantially decomposed by water, (ii) a base, (iii) if desired, a stabilizing agent and/or a chelator and (iv) if desired one or more inert pharmaceutically acceptable carriers and/or formulating agents and/or adjuvants, at least one of said ingredients (i) to (iv) being stored in a container having an atmosphere containing a sufficient amount of carbon monoxide, said ingredients (i) to (iv) optionally independently being combined, and (v) instructions for use with a prescription for reacting the ingredients of the kit with a metal selected from the group consisting of Mn, ^{99m}Tc, ^{186}Re or ^{188}Re in the form of a permetallate solution.

14. A kit for the preparation of a diagnostic or therapeutic pharmaceutical composition, comprising (i) a suitable substrate to be labelled with a metal selected from the group consisting of Mn, ^{99m}Tc, ^{186}Re or ^{188}Re, (ii) a reducing agent soluble in water but not substantially decomposed by water, (iii) a base, (iv) if desired, a stabilizing agent and/or a chelator, (v) if desired one or more inert pharmaceutically acceptable carriers and/or formulating agents and/or adjuvants, at least one of said ingredients (i) to (v) being stored in a container having an atmosphere containing a sufficient amount of carbon monoxide, said ingredients (i) to (v) optionally independently being combined, and (vi) instructions for use with a prescription for reacting the ingredients of the kit with said metal in the form of a permetallate solution.



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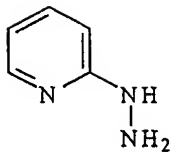

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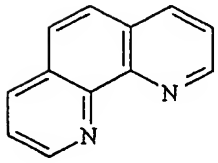

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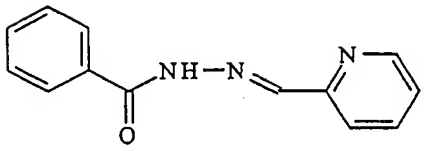

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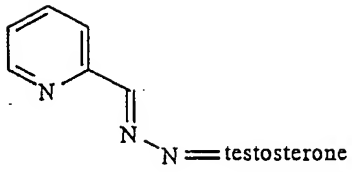

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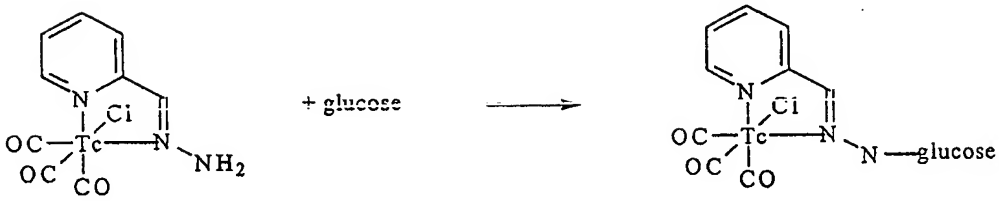

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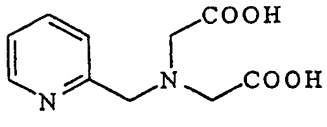

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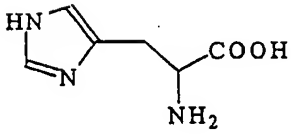

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Figure 1.

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/US 98/07979

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K51/04 C01G45/04 C07F13/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C01G C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BECK, WOLFGANG ET AL: "Metal complexes with biologically important ligands. XVIII. Histidinato-carbonyl complexes of molybdenum and tungsten" J. ORGANOMET. CHEM. (1980), 191(1), 73-7 CODEN: JORCAI;ISSN: 0022-328X, 1980, pages 73-77, XP002043518 see page 74 --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 June 1998	02.07.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Berte, M

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 98/07979

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BAMFORD, CLEMENT H. ET AL: "Evidence for the formation of the triaquatricarbonylmanganese(I) cation and related derivatives from pentacarbonylchloromanganese" J. CHEM. SOC., DALTON TRANS. (1978), (1), 4-8 CODEN: JCDTBI;ISSN: 0300-9246, 1978, pages 4-8, XP002043519 see page 4, column 1, paragraph 1 ---	1-14
A	VERONA, IVANA ET AL: "Regioselectivity in the nucleophilic functionalization of dibenzofuran, dibenzothiophene and xanthene complexes of Mn(CO)3+" J. ORGANOMET. CHEM. (1996), 524(1-2), 71-80 CODEN: JORCAI;ISSN: 0022-328X, 1996, XP002043520 see page 78, column 1, paragraph 3 ---	1
X	MEDER, HANS JOCHEN ET AL: "Metal complexes with biologically important ligands. XLII. Carbonyl metal complexes with anions of polyfunctional.alpha.-amino acids" Z. NATURFORSCH., B: ANORG. CHEM., ORG. CHEM. (1986), 41B(10), 1247-54 CODEN: ZNBAD2;ISSN: 0340-5087, 1986, XP002043521	2
Y	see page 1247, column 1, paragraph 3 ---	1-14
A	EP 0 105 785 A (CENTRE NAT RECH SCIENT) 18 April 1984 see claims ---	2-14
X	CHEMICAL ABSTRACTS, vol. 126, no. 18, 5 May 1997 Columbus, Ohio, US; abstract no. 245901, EGLI, ANDRE ET AL: "Hydrolysis of the Organometallic Aqua Ion fac-Triaquatricarbonylrhenium(I). Mechanism, pKa, and Formation Constants of the Polynuclear Hydrolysis Products" XP002043522 see abstract & ORGANOMETALLICS (1997), 16(9), 1833-1840 CODEN: ORGND7;ISSN: 0276-7333, 1997, ---	1

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INTERNATIONAL SEARCH REPORT

Inter. ..onal Application No
PCT/US 98/07979

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SCHBIGER P.A. ET AL.: "Versatilty of the "fac-Tc(CO)3" (Tc-99m) moiety for the labeling of various biomolecules." JOURNAL OF NUCLEAR MEDICINE, ABSTRACT BOOK, May 1997, NEW YORK US, page 180P XP002068168 see abstract N0774 -----	1-14

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 2-14

In view of the large number of compounds, which are defined by the general definition in the independent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, Chapter III, paragraph 2.3).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/07979

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2-14
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. al Application No
PCT/US 98/07979

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0105785 A	18-04-1984	FR 2533570 A	30-03-1984
		CA 1220469 A	14-04-1987
		CA 1234037 C	15-03-1988
		DE 3375788 A	07-04-1988
		JP 59080695 A	10-05-1984
		US 4983646 A	08-01-1991
		US 4656142 A	07-04-1987